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Award Number: DAMD17-98-1-8515

TITLE: Influence of Bone Remodeling Inhibition on the Development of Experimental Stress Fractures

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REPORT DATE: September 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Annual (1 Sep 01 - 31 Aug 02)

4. TITLE AND SUBTITLE
Influence of Bone Remodeling Inhibition on the Development of Experimental Stress Fractures

5. FUNDING NUMBERS
DAMD17-98-1-8515

6. AUTHOR(S):
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Case Western Reserve University
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Stress fractures result from repetitive loading and have been regarded as a mechanical fatigue-driven process. However, a number of studies indicate that increased remodeling precedes the occurrence of bone microdamage and stress fractures, suggesting a central role for bone remodeling in the pathogenesis of stress fractures. Our ongoing experiments test the hypothesis by pharmacological inhibition of bone remodeling will slow the subsequent accumulation of microdamage, diminishing the severity of the stress fracture. We are using a bisphosphonate (BIS) in the rabbit tibial stress fracture model, to test the hypothesis that reactive remodeling within the cortex drives the development of stress fractures. Results to date indicate that BIS antiresorptive therapy reduces the intensity of the stress fracture response, as indicated by ^{99m}Techneitum bone scans, with the uptake of ^{99m}Techneitum reduced by approximately 50 percent in treated animals as compared to saline-treated controls. However, BIS treatment attenuated, but did not completely prevent the stress fracture response. These data are consistent with the hypothesis that bone remodeling contributes to the pathogenesis of stress fracture. The implication of this suppression on the later accumulation of bone microcracks and the evolution of final stress fracture are unknown are currently under investigation.

14. SUBJECT TERMS	15. NUMBER OF PAGES
stress fractures, bone remodeling	10
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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ADMINISTRATIVE INTRODUCTION

Because the continuing lag in completing the animal portion of these experiments, we requested and were granted a no cost extension for year 3 of the grant, through June, 2002. This lag resulted from continuing staffing and equipment problems at the Henry Ford Hospital. In addition, we (Mount Sinai School of Medicine) negotiated a subcontract from the Henry Ford Hospital, to allow us to begin histological processing of the large specimen backlog that accrued at the Henry Ford Hospital as a result of personnel loss. We recruited a post-doctoral fellow, Dr. Liyun Wang, to Mount Sinai to work full time on the analyses of specimens. Finally, at the end of June 2002, we began the process of transferring the remainder of the grant solely to Mount Sinai to complete the analyses of stress fracture bone samples.

INTRODUCTION

Stress fractures result from repetitive loading and have been regarded as a mechanical fatigue-driven process. However, histopathological data and experimental data from our laboratory suggests that increased remodeling precedes the occurrence of bone microdamage and stress fractures, suggesting a central role for increased intracortical remodeling in the pathogenesis of stress fractures. Thus, we propose that stress fracture occurs through a positive feedback mechanism, in which increased mechanical usage stimulates focal bone turnover, resulting in a local increase in porosity. Microdamage accumulation and stress fractures result from continued cyclic loading of this transiently osteoporotic bone. These experiments test the hypothesis by pharmacologically inhibiting the bone remodeling response; the subsequent accumulation of microdamage and the severity of the stress fracture can be diminished. This hypothesis has been tested experimentally in the rabbit tibial stress fracture model, which was developed in our laboratory. To test the hypothesis that reactive remodeling within the cortex drives the development of stress fractures, the effect of remodeling suppression using a bisphosphonate on the accumulation of bone microdamage and diminishing the severity of stress fracture will be examined. To date, outcomes of these experiments have been assessed using bone scintigraphy; histomorphometry and biomechanical studies are ongoing.

SUMMARY OF RESEARCH

Our objectives in these experiments are to use the rabbit tibial stress fracture model:

1. to determine at the whole bone level whether bisphosphonate inhibition of intracortical remodeling attenuates the increase in focal bone ^{99m}Technetium uptake which characterizes the development of stress fracture,
2. to determine at the tissue level whether bisphosphonate inhibition of intracortical remodeling decreases the accumulation of cortical bone microdamage which occurs at the site of stress fracture, and
3. to determine how stress fracture compromises mechanical properties of long bones and whether pharmacological inhibition of remodeling can offset that functional deficit.

The project is proceeding toward the goals originally outlined for Year 3, with all procedures continuing. Because the continuing lag in completing the animal portion of these experiments, we requested and were granted a no cost extension for year 3 of the grant, through June, 2002. This lag resulted from continuing staffing and equipment problems at the Henry Ford Hospital.

We implemented the following alternative plan to begin to remediate the large backlog of specimens and to accomplish the key goals of our research program:

- In September,2001, we negotiated a subcontract from the Henry Ford Hospital, to allow us to begin histological processing and analyses of specimens in Dr. Schaffler's laboratory, at the Mount Sinai Medical Center in New York.

- We recruited a post-doctoral fellow, Dr. Liyun Wang, to Mount Sinai to work full time on the analyses of specimens. She began work in February 2002

- All bone specimens, data records, ^{99m}Technetium bone scans and radiographs have been transferred from the Henry Ford Hospital to the Dr. Schaffler at the Mount Sinai School of Medicine

- At the end of June 2002, we began the process of transferring the remainder of the grant solely to Mount Sinai to complete the analyses of stress fracture bone samples. This grant transfer is pending.

RESULTS

^{99m} Technetium bone scans

We have completed the preliminary analyses of ^{99m}Technetium bone scans of the reaction sites in experimentally loaded and non-loaded control tibiae.

Methods: The following procedure was used for ^{99m}Technetium injection, scanning, and quantification to control for variability between animals and among groups. The animals were each injected with 3 mCurie of ^{99m}Tc starting at 2:00 PM in a predetermined sequence. The isotope was administered IV in the ear vein, with an injection time of about 5-6 minutes per rabbit. Scans were conducted 3 hours later to image the bone phase of ^{99m}Technetium. The rabbits were scanned using a General Electric STARCAM System with a pinhole collimator and the data archived on optical disk for later analysis. Prior to scanning, rabbits were anesthetized with ketamine -xylazine and the lower extremities placed into one of two positioning devices. The anterior positioning device captured the lower leg at the distal tibia and positioned the legs with the anterior aspect of the leg toward the collimator. The lower limbs are slightly separated, parallel, and level. The positioning for the medial view places the lower extremity of the animal in a device that positions the legs at a 60° angle and level to the collimator. The total time to obtain both A-P and M-L images of each rabbit was about 12-15 minutes. A standardized area was used to determine a region of interest at the stress fracture site for the anterior view. This region of interest was of the same dimensions for all animals and provides an average count per pixel of isotope incorporation within the standard area. The same standardized area was also used to determine a background level of isotope incorporation. This region is distal to the site of stress fracture and also had the same dimensions for all animals. An average count of isotope incorporation per pixel was obtained within the background area. The average counts per pixel in the stress fracture region of interest were normalized by dividing by the average counts per pixel in the background region (*Average Counts per Pixel Stress Fracture Region of Interest/Average Counts per Pixel Background Region*). The mean value for each time period was compared between the bisphosphonate-injected and saline-injected groups using t-test. Differences among groups over time were assessed using one-way ANOVA.

^{99m}Technetium bone scans and analyses were successfully completed on 92 of 124 animals used on the experiment. These are distributed as follows:

- 3-week loaded animals (n=16)
- 6-week loaded animals (N=60)
- non-loaded, control animals (n=16).

In addition, for 6-week loaded animals, sequential bone scans we performed such that each animal was scanned at baseline (before loading), and then after 3 and 6 weeks of loading, to allow us to assess the progress of the lesions. Only animals for which there will be correlated histological studies underwent bone scans; animals whose tibiae were destined for biomechanical studies (n=32 animals, distributed among all experimental

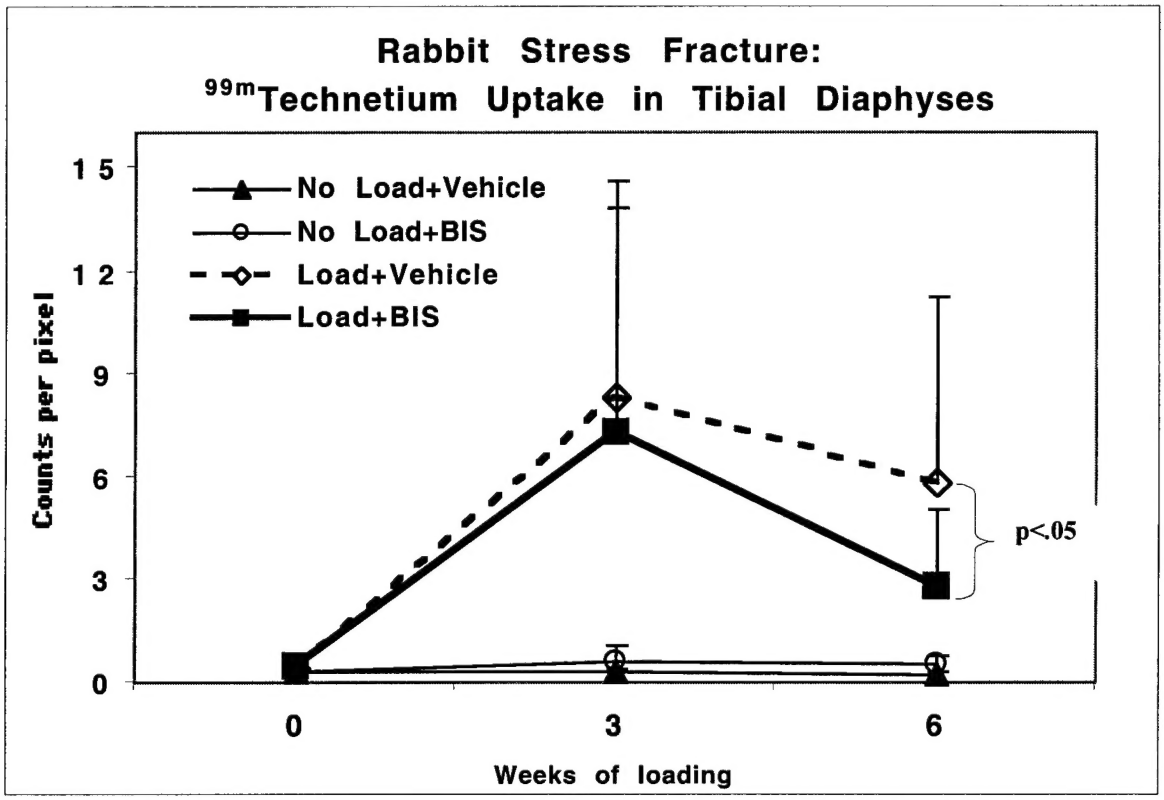
groups) were not subjected to bone scans. Animal groups and analytical procedures are summarized in Table 1.

Table 1: Summary of loading groups and analytical procedures:

	ANALYTICAL PROCEDURE		
	^{99m}Tc bone scans	**Histology**	**Biomechanics**
Non-loaded animals (n=26)			
13 BIS-treated	0, 3 & 6 weeks	pending	pending
13 saline-treated	(n=16)	(n=16)	(n=10)
3-week loaded animals (n=16)			
8 BIS-treated	0 & 3weeks	pending	none
7 saline-treated	(n=15)	(n=15)	
6 week loaded animals (n=82)	0, 3 & 6 weeks	pending	pending
40 BIS-treated	(n=60)	(n=60)	(n=22)
42 saline-treated			

Results: Tibiae in animals receiving antiresorptive treatment using bisphosphonate (BIS) showed reduced intensity of the stress fracture response, as indicated by ^{99m}Technetium bone scans after both 3 and 6 weeks of daily loading. At 3 weeks, ^{99m}Tc uptake was approximately 10 percent lower in treated animals, this difference was not significant ($p > 0.4$). After 6 weeks of loading, ^{99m}Tc uptake was significantly lower (approximately 50 percent, $p < .05$) in bisphosphonate-treated animals than in non-treated animals. Nevertheless, ^{99m}Tc uptake at 6 week was still significantly ($p < .001$) higher than in non-loaded, bisphosphonate-treated animals. These data are summarized in Figure 1.

Figure 1



Reduction of bone technetium uptake in bisphosphonate-treated animals is consistent with our hypothesis that suppression of remodeling attenuates the severity of the stress fracture response. Suppression of remodeling did not completely eliminate the stress response of the bone. Both an "escape" from remodeling suppression, as well as periosteal reaction resulting from long-term loading, could both account for this finding. Resolution of this of tissue mechanism question awaits completion of the histological studies.

Histology studies:

Methods: Tibial diaphyses from all baseline, 3 week and 6 week loaded animals have been bulk-stained in either basic fuchsin or Villanueva's Bone stain. Bones were then embedded in methylmethacrylate. All blocks have been embedded and are in the process of being sectioned for histomorphometric analyses.

YEAR 3: Goals:

The goals of year 3 of the project were 1) to continue to mechanically load rabbit hind limbs (with and without pharmacological inhibition of remodeling) in order to complete mechanical loading experiments; 2) to complete the 99mTc bone scans and 3) to transfer of bone specimens to Dr. Schaffler's laboratory in New York to begin to remediate the back-log of specimens awaiting histomorphometry analyses.

KEY RESEARCH ACCOMPLISHMENTS: YEAR 3

Key finding: The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the stress fracture response, as indicated by ^{99m}Techneitum bone scans.

Note: *Our progress and accomplishments are revised from those originally projected in our statement of work.*

Because of a number of unanticipated problems encountered in the Henry Ford component of this grant, the project has been running significantly behind our anticipated goals for this period. Delays in Year 1 were caused by a physical plant problem at the Henry Ford animal facility, which resulted in more than 6 months delay in the start of work on this project. These delays were detailed in our Year 1 progress report. Because experimental loading take several weeks to complete and relies on one key piece of equipment, this initial delay propagated through the project, even as we endeavor to catch up. We also note that we have had to deal with turnover in key technical personnel, which has further slowed our progress.

During the past year, we have made great progress to begin to remediate the large backlog of specimens and to achieve the key goals of the project. We have accomplished the following:

- Subcontract from the Henry Ford Hospital to allow us to begin histological processing and analyses of specimens in Dr. Schaffler's laboratory, at the Mount Sinai Medical Center in New York.

- We recruited a post-doctoral fellow, Dr. Liyun Wang, to Mount Sinai to work full time on the analyses of specimens. She began work in February 2002

- All animal studies have been completed.

Progress Report

- All bone specimens, data records, ^{99m}Technetium bone scans and radiographs have been transferred from the Henry Ford Hospital to the Dr. Schaffler at the Mount Sinai School of Medicine

- Analyses of all ^{99m}Technetium bone scans have been completed

- All tibias (n=83 pairs) have been bulk-stained, embedded and are currently being sectioned for histological study

- Design for biomechanical testing apparatus has been initiated

- At the end of June 2002, we began the process of transferring the remainder of the grant solely to Mount Sinai to complete the analyses of stress fracture bone samples. This grant transfer is pending.

REPORTABLE OUTCOMES

The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the bone response to repetitive stress, as indicated by ^{99m}Techneitum bone scans.

CONCLUSIONS

None to date. Experiments are ongoing.